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Minimizing the effect of precipitation in clarifying the responses of leaf N and P stoichiometry to temperature☆

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ABSTRACT

How terrestrial ecosystem responds to global warming has received wide attention. Plant stoichiometry has the potential to reflect ecosystem responses to climate change, thus, investigating the variations in plant stoichiometry with temperature is important and necessary for revealing the responses of terrestrial ecosystem to global warming. Although many studies had explored the relationships between plant N, P stoichiometry and temperature, previous field investigations did not eliminate the interference of precipitation effect with these observed relationships. To minimize the effect of precipitation on leaf N, P stoichiometry, this investigation was conducted across a temperature gradient over broad geographical scale along the 400 mm isohyet, which extends about 6000 km in China. This study showed that leaf N did not vary, whereas leaf P decreased and leaf N:P ratio increased with increasing mean annual temperature (MAT). The responses of leaf N and P stoichiometry to MAT observed in this study might be general patterns; because they were almost ubiquitous across functional groups, genera and species examined, and moreover, they were independent of vegetation and soil type. It could be inferred from this study that global warming in future will have no effect on leaf N, but reduce leaf P and increase leaf N:P ratio. Stable leaf N and varied leaf P with changing MAT suggested that leaf N and P decoupled with changing temperature.

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1. Introduction

Global warming has caused many serious problems to the ecosystem, and this has gained widespread attentions. Biological stoichiometry is the theory of studying dynamic balance of nutrients in organisms (Elser et al., 2000a; Sterner and Elser, 2002; Anderson et al., 2004; Güsewell, 2004), and it reflects the diversified relationships between organisms and environment (Tao et al., 2018). Thus, it is a useful indicator in understanding responses of ecosystems to global warming (Elser et al., 2010; Sardans et al., 2012; Yue et al., 2017).

Field manipulation experiments have greatly improved our understanding of dynamics of plant stoichiometry under climate warming scenario (An et al., 2005; Dijkstra et al., 2012; Velthuis et al., 2017; Zhang et al., 2017), but it remains unknown whether the observed results are realistic in natural ecosystems that experience ongoing global warming. Thus, field investigations addressing the underlying patterns of stoichiometric shifts across a temperature gradient over broad geographical scales are necessary.

There are numerous filed investigations conducted at global scales (Reich and Oleksyn, 2004; Wright et al., 2004), regional scales (Han et al., 2005, 2011; He et al., 2006; Zhang et al., 2012; Sardans et al., 2016; Tang et al., 2018), and even local sites (Tan and Wang, 2016). For example, based on an analysis with a global data set, Reich and Oleksyn (2004) found that leaf N was non-linearly correlated with mean annual temperature (MAT) and the turning point was about 5–10 °C; while leaf P generally decreased and leaf N:P ratio increased with MAT. Han et al. (2011) collected data from his field measurements and previous published papers (totally 752

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sites across China), and found that both leaf N and P were negatively correlated with MAT, while leaf N:P ratio did not vary with temperature. Tan and Wang (2016) measured leaf N and P concentrations in plants grown in Mount Gongga, and reported that as temperature decreased, plant N increased first, and then decreased, while plant P always kept a decreasing trend.

Many other environmental factors, such as precipitation, atmospheric N deposition, atmospheric carbon dioxide concentration, or light intensity, might also influence the relationships between leaf N and P stoichiometry and temperature. Among these factors, precipitation has been suggested to be the most important (Han et al., 2011). Several previous studies had attempted to eliminate the interference of precipitation effect based on mathematical method (Han et al., 2011). However, strictly speaking, this problem had not been well solved and even rarely considered in the design of previous field investigations. Thus, we could be more reliable to clarify the responses of plant N, P stoichiometry to temperature if we design a field investigation where the effect of precipitation is minimized.

Previous studies have observed the decoupling of N and P under rapid climatic changes (Delgado-Baquerizo et al., 2013; Yuan and Chen, 2015; Tan and Wang, 2016). The N cycle in terrestrial ecosystem is mainly controlled by biological processes like biological N fixation. Conversely, the P cycle in terrestrial ecosystem is primarily associated with geochemical processes, such as rock weathering (Aerts and Chapin, 2000). In general, the coupled relationship of N and P are maintained in terrestrial ecosystems. However, when facing rapid climate changes, this coupling could be broken due to different resistances of biological and geochemical processes to climate change. Hence, studying plant stoichiometric variations across a temperature gradient can also examine whether the correlation between N and P cycles become uncoupled with changing temperature.

An intensive field investigation was conducted across a temperature gradient over broad geographical scale along the 400 mm isohyet in China. The objective of the present study was to obtain more reliable patterns with respect to responses of plant N, P stoichiometry to temperature through minimizing the effect of precipitation changes. Meanwhile, this study also wanted to test whether the variations of N and P in plants were uncoupled with changing temperature.

2. Materials and methods

2.1. Site description

The 400 mm isohyet, which is the dividing line between semi-humid and semi-arid region, extends about 6000 km in China. In this study, we set up a transect along the isohyet from Luoguhecun (site 1, 53°29′N, 122°15′E) in Heilongjiang Province, northeast China to Naqu (site 58, 31°41′N, 91°96′E) in Tibet Autonomous Region, southwest China. There are 58 sampling sites along the transect (Fig. 1). Among these sampling sites, Qumalai has the lowest MAT of −5.1 °C, while Hengshan-1 has the highest MAT of +9.7 °C. The average of mean annual precipitation (MAP) of sampling sites is 397.24 mm. Site information is summarized in Table S1.

2.2. Plant sampling and chemical analysis

The plant sampling was conducted in the summers of 2008 and 2013 respectively. Sample sites were chosen far away from the cities to avoid the influence of anthropic activities. At each site, all plant species with high abundance in the local community or wide distribution along the transect were collected. 5–7 of individuals from each species were sampled and the same number of leaves was collected from each individual. For herbs and shrubs, the uppermost healthy and mature leaves of each individual were collected. For tree species, eight leaves with four cardinal directions (two leaves at each direction) above 8–10 m ground were sampled (see detailed method in Wang et al., 2013). There was a total of 658 plant samples along this transect.

All samples were cleaned out in ultrasonic cleaner, followed by oven drying at 65 °C, and then ground into fine powders. Leaf N concentrations of all samples were measured using an elemental analyzer (vario macro, elementar, Germany); leaf P concentrations were analyzed by ICP-OES (ICP-OES 7300DV, PerkinElmer, USA) after HNO_3-H_2O_2 digestion in the digester (MARS Xpress, CEM, USA). Leaf N and P concentration was expressed as g kg^{-1} on dry mass basis, and the N:P ratio in samples were calculated on a mass basis.

2.3. Statistical analysis

To improve data normality, Leaf N and P concentrations and N:P ratio were ln-transformed before doing regression analyses. Bivariate correlation analyses of leaf N, P and N:P ratio vs. MAT were performed to obtain the relationships between leaf N, P and N:P ratio and temperature. Partial correlation analyses were conducted to detect the influences of vegetation and soil type on the relationships between leaf N and P stoichiometry and temperature. To explore the influences of climate, vegetation type and soil type on leaf N and P stoichiometry, we performed multiple regressions in which MAT, MAP and altitude were used as quantifiable independent variables, vegetation and soil type as qualitative independent variables, and leaf N and P stoichiometry as dependent variables. Considering the strong relationship between vegetation and soil type, vegetation type and soil type were separately introduced into the multiple regressions. All statistical analyses were performed using the software of SPSS 20.0 (IBM Corporation, USA), with significance being determined at the 0.05 level.

3. Results

3.1. Variations of leaf N and P stoichiometry across the temperature gradient for all species pooled together

For all species pooled together, leaf N and P concentrations ranged from 2.9 to 207.9 g kg^{-1} and 0.13–25.63 g kg^{-1}, with an average of 26.3 g kg^{-1} and 2.26 g kg^{-1}, respectively. Leaf N:P ratio ranged from 0.1 to 122.9, with an average of 14.7. Bivariate correlation analyses showed that leaf N was not related to MAT ($r = 0.02$, $p > 0.05$) (Table 1 and Fig. 2a). Leaf P was negatively correlated with MAT ($r = -0.30$, $p < 0.001$) (Table 1 and Fig. 2b). Leaf N:P ratio was positively related to MAT ($r = 0.31$, $p < 0.001$) (Table 1 and Fig. 2c).

Partial correlation analyses, after controlling for vegetation type, soil type or the combined effect of vegetation and soil type respectively, yielded almost the same results as the bivariate correlation analyses although the correlation coefficients between leaf N and P stoichiometry and MAT in partial correlation analyses were slightly different from those in bivariate correlation analyses (Table 1).

3.2. Variations of leaf N and P stoichiometry in different plant functional groups across the temperature gradient

The leaf N concentrations in herbs, woody plants, annual herbs and perennial herbs ranged from 2.9 to 207.9 g kg^{-1}, 6.5–42.8 g kg^{-1}, 4.7–55.9 g kg^{-1} and 2.9–207.9 g kg^{-1}, with an average of 27.6 g kg^{-1}, 22.1 g kg^{-1}, 32.0 g kg^{-1} and 22.0 g kg^{-1}, respectively. Leaf N showed significant differences between herbs and woody plants ($p = 0.005$),

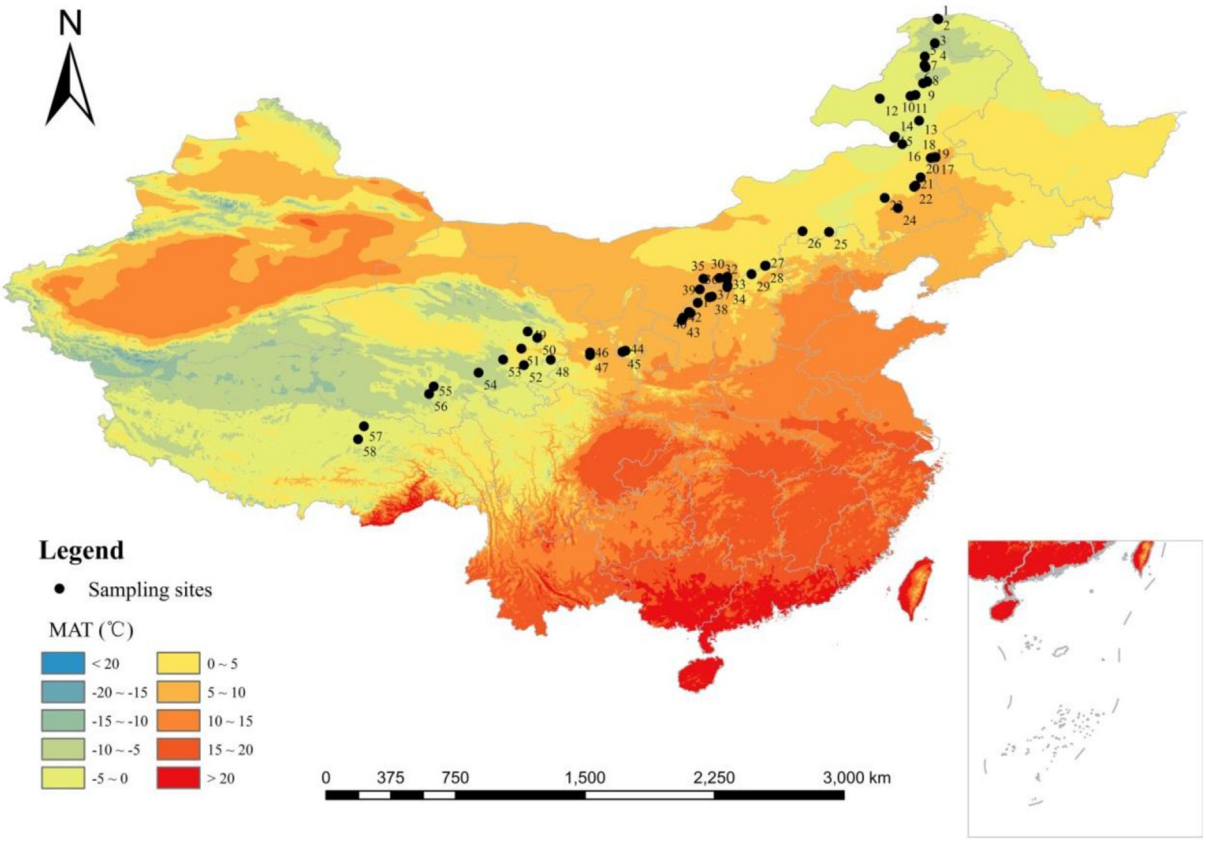

Fig. 1. Sketch of sampled region. Sample sites are indicated with numbers. Detailed information of the sites is showed in Table S1.

Table 1
Comparison of the results of bivariate correlation analysis of leaf N and P stoichiometry in all plant samples vs. temperature with that of partial correlation analyses as vegetation type, soil type and the combined effect of vegetation type and soil type were controlled for, respectively.

Variable controlled for	N		P		N:P	
	r	*p*	r	*p*	r	*p*
None†	0.02	ns	−0.30	***	0.31	***
Vegetation type#	0.02	ns	−0.18	***	0.22	***
Soil type$	0.01	ns	−0.12	**	0.13	**
Combined influence&	0.02	ns	−0.15	***	0.19	***

Note: r is correlation coefficient; *p* is significance of correlation analysis; **, *** and ns mean $p < 0.01$, $p < 0.001$ and $p > 0.05$, respectively; † is the bivariate correlation between leaf N and P stoichiometry and temperature; #, $ and & represent the partial correlations of leaf N and P stoichiometry and temperature as vegetation type, soil type and the combined effect of vegetation and soil type were controlled for, respectively.

and between annual and perennial herbs ($p < 0.001$) (Fig. S1a, d). The leaf P concentrations in herbs, woody plants, annual herbs and perennial herbs ranged from 0.13 to 25.63 g kg^{-1}, 0.37–5.95 g kg^{-1}, 0.27–10.12 g kg^{-1} and 0.13–25.63 g kg^{-1}, with an average of 2.45 g kg^{-1}, 1.62 g kg^{-1}, 2.71 g kg^{-1} and 2.17 g kg^{-1}, respectively. Significant differences in leaf P between herbs and woody plants ($p = 0.003$), and between annual and perennial herbs ($p = 0.005$) were observed (Fig. S1b, e). The leaf N:P ratios in herbs, woody plants, annual herbs and perennial herbs ranged from 0.1 to 122.9, 4.1 to 72.1, 0.1 to 68.8 and 0.7 to 122.9, with an average of 14.0, 17.0, 13.5 and 14.7, respectively. One-way ANOVA analyses revealed that there was a significant difference in leaf N:P ratios between herbs and woody plants ($p = 0.035$), and no difference between annual and perennial herbs ($p = 0.19$) (Fig. S1c,f).

Although leaf N, P, and even leaf N:P ratios varied across functional groups, the patterns of variations in leaf N, P and N:P ratios with MAT was independent of functional groups. When MAT increased, leaf N always kept stable ($p > 0.05$ for all functional groups), whereas leaf P decreased ($p < 0.001$, 0.01, 0.001 and 0.01 for herbs, woody plants, annual herbs and perennial herbs, respectively) and leaf N:P ratios increased for all functional groups ($p < 0.001$for herbs, $p = 0.001$ for woody plants, $p < 0.001$ for annual herbs and $p = 0.001$ for perennial herbs) (Fig. S2a-l).

3.3. Variations of leaf N and P stoichiometry in *Artemisia* and *Chenopodium* across the temperature gradient

Artemisia and *Chenopodium* were two most widely distributed genera across the temperature gradient. To further reveal the responses of leaf N and P stoichiometry to temperature, we examined the variations of N and P in the two genera with changing temperature. The leaf N concentrations in *Artemisia* and *Chenopodium* ranged from 8.4 to 207.9 g kg^{-1} and 13.9–55.9 g kg^{-1}, with an average of 25.6 g kg^{-1} and 40.6 g kg^{-1}, respectively. There was a significant difference in leaf N between *Artemisia* and *Chenopodium* ($p < 0.001$) (Fig. S1g). The leaf P concentrations in *Artemisia* and *Chenopodium* ranged from 0.46 to 6.48 g kg^{-1} and 0.93–75.04 g kg^{-1}, with an average of 2.82 g kg^{-1} and 3.97 g kg^{-1}, respectively. The difference in leaf P was not significant between the two genera ($p > 0.05$) (Fig. S1h). The leaf N:P ratios in *Artemisia* and *Chenopodium* ranged from 3.1 to 57.7 and 0.5 to 29.0, with an average of 11.3 and 15.8, respectively. A significant difference in N:P ratios was observed between *Artemisia* and *Chenopodium* ($p < 0.001$) (Fig. S1i).

Although the two genera showed significant differences in leaf N and N:P ratios, their N, P stoichiometry responded increasing

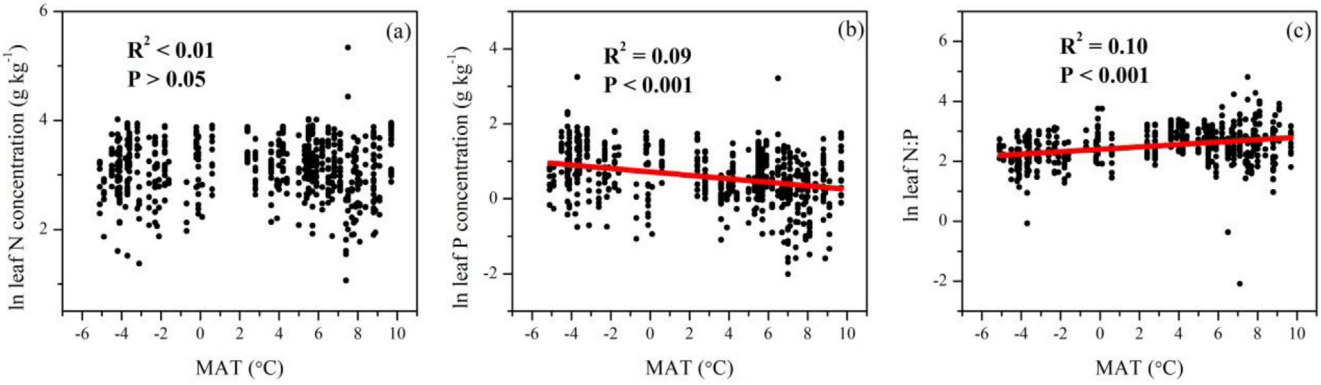

Fig. 2. Variations of leaf N and P concentrations and N:P ratio in all plant samples across the temperature gradient.

MAT in the same or similar way (Fig. S3a-f). The leaf N in both *Artemisia* and *Chenopodium* kept stable with increasing MAT ($p > 0.05$ for both genera) (Fig. S3a, d). The leaf P in both *Artemisia* and *Chenopodium* decreased ($p < 0.05$ for *Artemisia*, $p < 0.01$ for *Chenopodium*), whereas the leaf N:P ratios in the two genera showed significant increasing trends with MAT ($p < 0.05$ for *Artemisia*, $p = 0.001$ for *Chenopodium*) (Fig. S3b,c,e,f).

3.4. Variations of leaf N and P stoichiometry in Plantago depressa, Setaria viridis and Amaranthus retroflexus across the temperature gradient

To further explore the responses of leaf N and P stoichiometry to temperature, we examined the variations of N and P stoichiometry in three of the most widely distributed species, *Plantago depressa, Setaria viridis and Amaranthus retroflexus*, across the temperature gradient. The leaf N in *Plantago depressa*, *Setaria viridis* and *Amaranthus retroflexus* ranged from 12.0 to 48.6 g kg^{-1}, 4.7–55.2 g kg^{-1} and 21.6–52.3 g kg^{-1}, with an average of 27.3 g kg^{-1}, 22.7 g kg^{-1} and 38.4 g kg^{-1}, respectively. Leaf N showed a significant variation across the three species ($p < 0.001$) (Fig. S1j). The leaf P in *Plantago depressa*, *Setaria viridis* and *Amaranthus retroflexus* ranged from 0.98 to 6.73 g kg^{-1}, 0.27–3.40 g kg^{-1} and 1.90–10.12 g kg^{-1}, with an average of 2.76 g kg^{-1}, 1.70 g kg^{-1} and 3.92 g kg^{-1}, respectively. Species showed a significant influence on leaf P ($p < 0.001$) (Fig. S1k). The leaf N:P ratios in *Plantago depressa*, *Setaria viridis* and *Amaranthus retroflexus* ranged from 2.6 to 24.6, 0.1 to 68.8 and 3.8 to 18.4, with an average of 11.2, 15.4 and 10.7, respectively. Leaf N:P ratios depended on species ($p < 0.001$) (Fig. S1l).

The three species showed the same or similar patterns in the responses of leaf P to MAT, however, the responses of leaf N and N:P ratios to MAT were not identical. Leaf P in the three plant species decreased with MAT ($p < 0.001$ for *Plantago depressa*, $p < 0.05$ for *Setaria viridis* and $p < 0.01$ for *Amaranthus retroflexus*) (Fig. S4b, e, h). Leaf N in both *Setaria viridis* and *Amaranthus retroflexus* remained constant ($p > 0.05$ for both) but increased in *Plantago depressa* with increasing MAT ($p = 0.001$) (Fig. S4a, d, g). Leaf N:P ratios in both *Plantago depressa* and *Amaranthus retroflexus* increased ($p < 0.001$ for *Plantago depressa* and $p = 0.001$ for *Amaranthus retroflexus*) but kept unchanged in *Setaria viridis* with MAT ($p > 0.05$) (Fig. S4c, f, i).

3.5. Influences of MAT, MAP, altitude, vegetation type and soil type on leaf N and P stoichiometry

Multiple regression of Model 1 suggested that only 4% of the variability in leaf N in all plant samples could be explained by MAT, MAP and altitude ($R^2 = 0.04$, $p < 0.001$) (Table 2). Compared to the Model 1, multiple regressions with vegetation and soil type as dummy variables showed a slight increase in explanation degree ($R^2 = 0.08$, $p < 0.001$ for the model with vegetation and $R^2 = 0.09$, $p < 0.001$ for the model with soil) (Table 2).

MAT, MAP and altitude together contributed 21% of the variability in leaf P in all plant samples ($R^2 = 0.21$, $p < 0.001$). When vegetation type was taken into consideration, a greater explanation degree in leaf P variance was observed ($R^2 = 0.25$, $p < 0.001$) (Table 2). When soil type was introduced into the analysis, the R^2-value also increased ($R^2 = 0.24$, $p < 0.001$) (Table 2).

The combined explanation of MAT, MAP and altitude for variability in leaf N:P ratio in all plant samples was 17% ($R^2 = 0.17$, $p < 0.001$) (Table 2). When vegetation type was incorporated into the model, the degree of interpretation of the variability in leaf N:P increased to 21% ($R^2 = 0.21$, $p < 0.001$) (Table 2). Multiple regression of Model 3 revealed that soil type could also account for the increased variability in leaf N:P ratio ($R^2 = 0.20$, $p < 0.001$) (Table 2).

4. Discussion

For all species pooled together, this study showed that leaf N did not vary with MAT. Furthermore, the four functional groups and the two widespread genera investigated in the present study also exhibited the same pattern in the variation in N with MAT. Besides, among the three widespread species, *Amaranthus retroflexus and Setaria viridis* both showed N stability with changing MAT. Thus, this study suggested that the pattern of being stable in leaf N with changing MAT might be a common phenomenon. In addition, partial correlation analyses, in which vegetation and soil type were controlled for, exhibited almost the same results as bivariate correlation analyses (Table 1). This indicated that the pattern of leaf N vs. MAT was independent of vegetation type and soil type. Thus, stable leaf N concentration associated with temperature should be a general pattern.

Table 2
Results from multiple regressions of leaf N and P stoichiometry in all plant samples against MAT, MAP, altitude, vegetation type and soil type.

	Model-1		Model-2		Model-3	
	R^2	p	R^2	p	R^2	p
N	0.04	***	0.08	***	0.09	***
P	0.21	***	0.25	***	0.24	***
N:P	0.17	***	0.21	***	0.20	***

Note: Model-1, Model-2 and Model-3 are the multiple regressions of leaf N and P stoichiometry in all plant samples against MAT, MAP and altitude, against MAT, MAP, altitude and vegetation type, and against MAT, MAP, altitude and soil type, respectively. *** means $p < 0.001$.

Except being consistent with the founding of Yang et al. (2016) that leaf N in shrub species remained constant with MAT in northern China, our observation was different from the results in most previous researches (Reich and Oleksyn, 2004; Han et al., 2005; Kang et al., 2011; Chen et al., 2013; Sardans et al., 2015; Tang et al., 2018). Han et al. (2005) collected data from the published literature and reported that leaf N decreased with increased MAT. Kang et al. (2011) obtained a data set of nutrient concentrations of Norway spruce and found that leaf N varied non-linearly with MAT and the peak of N concentration occurred at 6–8 °C. Chen et al. (2013) measured leaf N and P concentrations of 386 woody species across eastern China and showed that leaf N was negatively related to MAT. Sardans et al. (2015) analyzed leaf N and P concentrations and its ratio in 50 tree species across Europe and concluded that leaf N was also negatively correlated with MAT. Precipitation has been consistently suggested to one important influential factor on leaf N; and Han et al. (2011) and Zhao et al. (2016) both even argued that precipitation explained more variation than temperature for leaf N. However, most previous studies were unable to effectively eliminate the interference of precipitation effect. Thus, this could result in our result differing from the observed patterns in previous studies.

Constant concentration of leaf N across the temperature gradient might reflect the neutralization between two mechanisms, the temperature-plant physiological hypothesis (TPPH) and the biogeochemical hypothesis (BH). The TPPH proposes that plants could improve their nutrient status to offset the decrease of their metabolic rates caused by low temperature (Woods et al., 2003; Reich and Oleksyn, 2004). As a consequence, leaf N decreases with increased temperature. The BH suggests that soil N mineralization is suppressed by low temperature, which results in the decreased soil N availability (McGroddy et al., 2004; Reich and Oleksyn, 2004). Since leaf N is controlled by soil N availability, leaf N increases with increased temperature based on BH hypothesis.

When vegetation type and soil type were introduced into model, the explanation degree of the multiple regression increased to some extent (Table 2). Hence, it suggested that both vegetation type and soil type exerted a certain degree of influence on leaf N (Table 2). Considering that communities in which life-forms of the dominant plants was similar were generally treated as the same vegetation type, thus, vegetation type control of leaf N mainly reflected the effects of functional group on leaf N. Since leaf N had been reported to vary markedly across functional groups in the present study and previous studies (Kraft et al., 2008; Shi et al., 2012; Adler et al., 2014), it was not strange that the control of vegetation type on leaf N was observed in this study. As for soil type, different soil types would result in the distinctions in soil properties and soil microbial composition, and this could lead to soil type control of leaf N.

For all species pooled together, leaf P was negatively correlated with MAT. Furthermore, the variances of leaf P with MAT showed the same pattern across the four plant functional groups, two widespread genera and three widespread species. Thus, it suggested that a negative relationship between leaf P and MAT might be a common result. In addition, partial correlation analyses revealed that the relationship between leaf P and MAT was not controlled by vegetation and soil type. Hence, we could conclude that negative relationship of leaf P-MAT should be a general pattern.

Although previous studies did not minimize the effect of precipitation on leaf P, they also reported a negative relationship between leaf P and MAT (Reich and Oleksyn, 2004; Han et al., 2005; Kang et al., 2011; Chen et al., 2013; Sardans et al., 2015; Yang et al., 2016; Tang et al., 2018). It seemed that the relationship between leaf P and MAT was not very relevant to the minimization of precipitation effect. Actually, this is not surprising because previous studies found that precipitation effect on leaf P was minor relative to temperature effect (Han et al., 2011; Zhao et al., 2016). Thus, whether or not precipitation effect is controlled for will not make a big difference in the relationship between leaf P and MAT.

The P source of plants is mainly from rock weathering, and thus the response of leaf P to changing temperature is not associated with the theory of BH. Meanwhile, Dijkstra et al. (2012) found that experimental warming could reduce soil P availability. Hence, a decrease in leaf P with increasing MAT could be the consequence of the combined influences of the TPPH mechanism and the reduction in soil P availability caused by high temperature. Multiple regression after vegetation type and soil type being introduced also showed an increase in the explanation degree for leaf P variances (Table 2), thus, indicating that both vegetation and soil type had an effect on leaf P. There could be analogical mechanisms responsible for the influences of vegetation and soil type on leaf P and leaf N. However, it needs further studies.

Leaf N:P ratio was observed to be positively related with MAT in the present study. This was consistent with the founding in most previous studies (Reich and Oleksyn, 2004; Kang et al., 2011; Chen et al., 2013; Sardans et al., 2015; Yang et al., 2016). This study also suggested that the positive relationship between leaf N:P ratio and MAT was a general pattern, because except *Setaria viridis*, all species pooled together, four functional groups, two genera and two species examined in the present study all exhibited the same pattern. Furthermore, partial correlation analyses showed that the relationship between leaf N:P ratio and MAT was independent of vegetation and soil type (Table 1).

The relationship between leaf N:P ratio and MAT was consistent with the expectation from Growth Rate Hypothesis (GRH). The GRH proposed that species under stress conditions often have conservative strategies and lower growth rates, and thus they could have higher N:P ratios (Sterner and Elser, 2002). The increased MAT along the 400 mm isohyet might result in decreased soil water availability (i.e. increased aridity), this would inhibit plant growth and lead to increased leaf N:P ratios. Hence, our field study provided a clear clue to the application of GRH in terrestrial ecosystems.

The arithmetic mean of leaf N:P ratio of all plant samples together in this study was 14.7, which was higher than that reported by Elser et al. (2000b) for 397 terrestrial plant species (leaf N:P ratio = l2.7), but lower than that reported by Han et al. (2005) for 753 plant species in China (leaf N:P ratio = 16.3). Leaf N:P ratio is an indicator of the relative limitation of N vs P. As N:P ratio is lower than 14, it indicates N limitation in terrestrial ecosystems; and when N:P ratio is greater than 16, it means P limitation (Koerselman and Meuleman, 1996). Hence, this study suggested that the most plants grown along the 400 mm isohyet were not under either N limitation or P limitation.

Yuan and Chen (2015) have reported that global climate changes could result in the decoupling of N and P in terrestrial plants. This study observed stable leaf N but varied leaf P with changing MAT, hence, there existed a decoupling of leaf N and P. The different controls of climate change on biological and geochemical processes could account for this decoupling because previous studies consistently suggested that N cycle is primarily associated with biological processes, whereas P cycle is mainly constrained by geochemical processes (Delgado-Baquerizo et al., 2013; Yuan and Chen, 2015; Tan and Wang, 2016).

5. Conclusion

This study addressed the responses of leaf N and P stoichiometry to temperature after minimizing the effect of precipitation. This

study showed that leaf N remained constant, whereas leaf P decreased and leaf N:P ratio increased with increasing MAT. Furthermore, the observed patterns were found to be almost ubiquitous across functional groups, genera and species examined in the present study. In addition, the patterns were independent of vegetation type and soil type. Thus, the phenomena of keeping leaf N constant, increasing leaf P and decreasing leaf N:P ratio with increasing MAT could be a general pattern. This study suggested that global warming could have no effect on leaf N, but decrease leaf P concentration and increase leaf N:P ratio. Since leaf N and leaf P responded changing MAT in two distinct ways, it resulted in a decoupling of N and P in plants associated with temperature.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.09.010.

References

Adler, P.B., Salguero-Gómez, R., Compagnoni, A., Hsu, J.S., Ray-Mukherjee, J., Mbeau-Ache, C., Franco, M., 2014. Functional traits explain variation in plant life history strategies. Proc. Natl. Acad. Sci. Unit. States Am. 111, 740–745.
Aerts, R., Chapin III, F.S., 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv. Ecol. Res. 30, 1–67.
Anderson, T.R., Boersma, M., Raubenheimer, D., 2004. Stoichiometry: linking elements to biochemicals. Ecology 85, 1193–1202.
An, Y., Wan, S., Zhou, X., Subedar, A.A., Wallace, L.L., Luo, Y., 2005. Plant nitrogen concentration, use efficiency, and contents in a tallgrass prairie ecosystem under experimental warming. Global Change Biol. 11, 1733–1744.
Chen, Y., Han, W., Tang, L., Tang, Z., Fang, J., 2013. Leaf nitrogen and phosphorus concentrations of woody plants differ in responses to climate, soil and plant growth form. Ecography 36, 178–184.
Delgado-Baquerizo, M., Maestre, F.T., Gallardo, A., Bowker, M.A., Wallenstein, M.D., Quero, J.L., Ochoa, V., Gozalo, B., Garcia-Gomez, M., Soliveres, S., Garcia-Palacios, P., Berdugo, M., Valencia, E., Escolar, C., Arredondo, T., Barraza-Zepeda, C., Bran, D., Carreira, J.A., Chaieb, M., Conceicao, A.A., Derak, M., Eldridge, D.J., Escudero, A., Espinosa, C.I., Gaitan, J., Gatica, M.G., Gomez-Gonzalez, S., Guzman, E., Gutierrez, J.R., Florentino, A., Hepper, E., Hernandez, R.M., Huber-Sannwald, E., Jankju, M., Liu, J., Mau, R.L., Miriti, M., Monerris, J., Naseri, K., Noumi, Z., Polo, V., Prina, A., Pucheta, E., Ramirez, E., Ramirez-Collantes, D.A., Romao, R., Tighe, M., Torres, D., Torres-Diaz, C., Ungar, E.D., Val, J., Wamiti, W., Wang, D., Zaady, E., 2013. Decoupling of soil nutrient cycles as a function of aridity in global drylands. Nature 502, 672–676.
Dijkstra, F.A., Pendall, E., Morgan, J.A., Blumenthal, D.M., Carrillo, Y., LeCain, D.R., Follett, R.F., Williams, D.G., 2012. Climate change alters stoichiometry of phosphorus and nitrogen in a semiarid grassland. New Phytol. 196, 807–815.
Elser, J.J., Sterner, R.W., Gorokhova, E.A., Fagan, W.F., Markow, T.A., Cotner, J.B., Harrison, J.F., Hobbie, S.E., Odell, G.M., Weider, L.J., 2000a. Biological stoichiometry from genes to ecosystems. Ecol. Lett. 3, 540–550.
Elser, J.J., Fagan, W.F., Denno, R.F., Dobberfuhl, D.R., Folarin, A., Huberty, A., Interlandi, S., Kilham, S.S., McCauley, E., Schulz, K.L., Siemann, E.H., Sterner, R.W., 2000b. Nutritional constraints in terrestrial and freshwater food webs. Nature 408, 578–580.
Elser, J.J., Fagan, W.F., Kerkhoff, A.J., Swenson, N.G., Enquist, G.J., 2010. Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. New Phytol. 186, 593–608.
Güsewell, S., 2004. N:P ratios in terrestrial plants: variation and functional significance. New Phytol. 164, 243–266.
Han, W., Fang, J., Guo, D., Zhang, Y., 2005. Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China. New Phytol. 168, 377–385.
Han, W.X., Fang, J.Y., Reich, P.B., Ian Woodward, F., Wang, Z.H., 2011. Biogeography and variability of eleven mineral elements in plant leaves across gradients of climate, soil and plant functional type in China. Ecol. Lett. 14, 788–796.
He, J.S., Fang, J., Wang, Z., Guo, D., Flynn, D.F., Geng, Z., 2006. Stoichiometry and large-scale patterns of leaf carbon and nitrogen in the grassland biomes of China. Oecologia 149, 115–122.
Kang, H., Zhuang, H., Wu, L., Liu, Q., Shen, G., Berg, B., Man, R., Liu, C., 2011. Variation in leaf nitrogen and phosphorus stoichiometry in Picea abies across Europe: an analysis based on local observations. For. Ecol. Manag. 261, 195–202.
Koerselman, W., Meuleman, A.F., 1996. The vegetation N: P ratio: a new tool to detect the nature of nutrient limitation. J. Appl. Ecol. 33, 1441–1450.
Kraft, N.J., Valencia, R., Ackerly, D.D., 2008. Functional traits and niche-based tree community assembly in an Amazonian forest. Science 322, 580–582.
McGroddy, M.E., Daufresne, T., Hedin, L.O., 2004. Scaling of C: N: P stoichiometry in forests worldwide: implications of terrestrial redfield-type ratios. Ecology 85, 2390–2401.
Reich, P.B., Oleksyn, J., 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. Proc. Natl. Acad. Sci. Unit. States Am. 101, 11001–11006.
Sardans, J., Rivas-Ubach, A., Peñuelas, J., 2012. The C: N: P stoichiometry of organisms and ecosystems in a changing world: a review and perspectives. Perspect. Plant Ecol. Evol. Systemat. 14, 33–47.
Sardans, J., Janssens, I.A., Alonso, R., Veresoglou, S.D., Rillig, M.C., Sanders, T.G., Carnicer, J., Filella, I., Farré-Armengol, G., Peñuelas, J., 2015. Foliar elemental composition of European forest tree species associated with evolutionary traits and present environmental and competitive conditions. Global Ecol. Biogeogr. 24, 240–255.
Sardans, J., Alonso, R., Carnicer, J., Fernández-Martínez, M., Vivanco, M.G., Peñuelas, J., 2016. Factors influencing the foliar elemental composition and stoichiometry in forest trees in Spain. Perspect. Plant Ecol. Evol. Systemat. 18, 52–69.
Shi, W., Wang, G., Han, W., 2012. Altitudinal variation in leaf nitrogen concentration on the eastern slope of Mount Gongga on the Tibetan Plateau, China. PLoS One 7 (9), e44628.
Sterner, R.W., Elser, J.J., 2002. Ecological Stoichiometry: the Biology of Elements from Molecules to the Biosphere (Princeton, NJ, USA).
Tan, Q., Wang, G., 2016. Decoupling of nutrient element cycles in soil and plants across an altitude gradient. Sci. Rep. 6, 34875.
Tang, Z., Xu, W., Zhou, G., Bai, Y., Li, J., Tang, X., Chen, D., Liu, Q., Ma, W., Xiong, G., He, H., He, N., Guo, Y., Guo, Q., Zhu, J., Han, W., Hu, H., Fang, J., Xie, Z., 2018. Patterns of plant carbon, nitrogen, and phosphorus concentration in relation to productivity in China's terrestrial ecosystems. Proc. Natl. Acad. Sci. U.S.A. 115, 4033–4038.
Tao, Y., Dan, D., Kun, L., Chengda, H., Haibing, C., Guo, F., Xu, Q., Sun, F., Wu, F., 2018. $\delta^{15}N$ and nutrient stoichiometry of water, aquatic organisms and environmental implications in Taihu lake, China. Environ. Pollut. 237, 166–173.
Velthuis, M., van Deelen, E., van Donk, E., Zhang, P., Bakker, E.S., 2017. Impact of temperature and nutrients on carbon: nutrient tissue stoichiometry of submerged aquatic plants: an experiment and meta-analysis. Front. Plant Sci. 8, 655.
Wang, G., Li, J., Liu, X., Li, X., 2013. Variations in carbon isotope ratios of plants across a temperature gradient along the 400 mm isoline of mean annual precipitation in north China and their relevance to paleovegetation reconstruction. Quat. Sci. Rev. 63, 83–90.
Woods, H.A., Makino, W., Cotner, J.B., Hobbie, S.E., Harrison, J.F., Acharya, K., Elser, J.J., 2003. Temperature and the chemical composition of poikilothermic organisms. Funct. Ecol. 17, 237–245.
Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M., Niinemets, Ü., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J., Villar, R., 2004. The worldwide leaf economics spectrum. Nature 428, 821–827.
Yang, X., Chi, X., Ji, C., Liu, H., Ma, W., Mohhammat, A., Shi, Z., Wang, X., Yu, S., Yue, M., Tang, Z., 2016. Variations of leaf N and P concentrations in shrubland biomes across northern China: phylogeny, climate, and soil. Biogeosciences 13, 4429–4438.
Yuan, Z.Y., Chen, H.Y., 2015. Decoupling of nitrogen and phosphorus in terrestrial plants associated with global changes. Nat. Clim. Change 5, 465–469.
Yue, K., Fornara, D.A., Yang, W., Peng, Y., Li, Z., Wu, F., Peng, C., 2017. Effects of three global change drivers on terrestrial C: N: P stoichiometry: a global synthesis. Global Change Biol. 23, 2450–2463.
Zhang, Q., Xie, J., Lyu, M., Xiong, D., Wang, J., Chen, Y., Li, Y., Wang, M., Yang, Y., 2017. Short-term effects of soil warming and nitrogen addition on the N: P stoichiometry of Cunninghamia lanceolata in subtropical regions. Plant Soil 411, 395–407.
Zhang, S.B., Zhang, J.L., Slik, J.W., Cao, K.F., 2012. Leaf element concentrations of terrestrial plants across China are influenced by taxonomy and the environment. Global Ecol. Biogeogr. 21, 809–818.
Zhao, N., Yu, G., He, N., Wang, Q., Guo, D., Zhang, X., Wang, R., Xu, Z., Jiao, C., Li, N., Jia, Y., 2016. Coordinated pattern of multi-element variability in leaves and roots across Chinese forest biomes. Global Ecol. Biogeogr. 25, 359–367.